

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A recrystallization inhibition assay method for determining recrystallization inhibition characteristics of a proteinaceous composition by a series of steps, the steps comprising:

- a) providing a test solution comprising a proteinaceous composition in a solvent;
- b) providing at least one control solution comprising at least one of the group: saline, phosphate buffered saline (PBS), and non-thermal hysteresis proteins (THP) containing proteinaceous control solutions;
- c) flash freezing said test solution and said control solution to obtain a frozen test solution and frozen control solution;
- d) raising the temperature of both said frozen test solution and said frozen control solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within said test solution;
- e) maintaining both said frozen test solution and said frozen control solution at the annealing temperature for a length of time sufficient to allow for ice recrystallization within said test solution;
- f) monitoring changes, by means of imaging, in ice crystal grain size in said test solution and said control solution over time; and
- g) quantitatively and statistically analyzing said imaging to determine said recrystallization inhibition characteristics;

wherein said determined recrystallization inhibition characteristics are defined by: relative concentration defined

by the concentration that is sufficient to exceed threshold of assay to known amounts of antifreeze protein extending to a concentration as low as between 0.5 $\mu$ g to 0.1 $\mu$ g per milliliter, and activity of thermal hysteresis proteins in a proteinaceous composition of said test solutions, by computation of a relative recrystallization inhibition (RI) factor, defined as the absolute value of the logarithm of the minimum THP dilution required to eliminate recrystallization inhibition activity, while reducing the effect of non-thermal hysteresis protein induced recrystallization inhibition effects, said reduction effect based upon measurement of said ice crystal size and computation of a relative recrystallization inhibition (RI) factor relative to said control solution.

Claim 2 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein said solvent is selected from at least one of the group consisting of water, saline, and phosphate buffered saline (PBS).

Claim 3 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein said control solution comprises one of the following: a solvent and a solution for non-specific recrystallization inhibition effects.

Claim 4 (cancelled)

Claim 5 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein said proteinaceous composition is selected from the group consisting of: purified Tm 12.86 and purified Tm 12.84.

Claim 6 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein said proteinaceous composition is selected from the group consisting of antifreeze polypeptides, antifreeze glycopeptides, recombinant antifreeze polypeptides, recombinant antifreeze glycopeptides, synthetic antifreeze polypeptides analogs, synthetic antifreeze glycopeptide analogs, cell culture products, activators, recombinant bacterial products, recombinant products, uncharacterized plant products and transgenic plant products.

Claim 7 (cancelled)

Claim 8 (previously amended): The recrystallization inhibition assay method as defined in claim 1 wherein said

proteinaceous composition comprises Tm 12.86 present in a concentration from about 0.5 ug/ml to about 25 ug/ml.

Claim 9 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein said proteinaceous composition has a total protein content less than or equal to 1 mg/ml in saline and phosphate buffered saline (PBS); and less than or equal to 0.005 mg/ml in water.

Claim 10 (cancelled)

Claim 11 (currently amended): The recrystallization inhibition assay method as defined in claim 1, wherein conditions to eliminate non-thermal hysteresis protein induced recrystallization inhibition conditions comprise saline -6°C for 30 min with a total protein content less than or equal to 1 mg/ml; or in water at -2°C for 2 hours with a total protein content less than or equal to 0.005 mg/ml.

Claim 12 (previously amended): The recrystallization inhibition assay method as defined in claim 11, under conditions that avoid hyperosmotic solutions.

Claim 13 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein changes to said ice crystal grain size over time is monitored by a method chosen from: photomicroscopy, digital or video imaging.

Claim 14 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein quantitative data is collected by measurement of the mean largest ice grain size for both said test and control solutions to provide a basis for numerical assessment of the extent of recrystallization inhibition occurring.

Claim 15 (previously amended): The recrystallization inhibition assay method as defined in claim 14, wherein composite mean largest grain size(mlgs) are obtained for said test solution and said control solution; which are then statistically compared.

Claim 16 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein quantitative data collection is collected by assessment using a densitometer of light transmitted through a low magnification full view photographic negative of frozen sample

wafer; absorbance peaks for said test solution is evaluated for maximum amplitude and statistically compared with said control solution.

Claim 17 (previously amended): The recrystallization inhibition assay method as defined in claim 1, wherein a dilution profile of said test solution is obtained over a wide dilution range until mean largest grain size (mlgs), or another quantifiably assessed response variable, are no longer significantly different from at least one of: saline, phosphate buffered saline (PBS), and non-temperature hysteresis proteins (THP) containing proteinaceous control solutions.

Claim 18 (previously amended) The recrystallization inhibition assay method as defined in claim 17, wherein composite mean largest grain size, or absorbance peak area (light scattering), or computer generated units (digital/video imaging) are calculated for said test solution and plotted as a function of the logarithm of sample concentration, with replicate dilution series tested, and compared to control solution baseline.

Claim 19 (previously amended): The recrystallization inhibition assay method as defined in claim 17, wherein linear regression analyses is used to approximate the linear portion of the dilution profile, with application of a transforming function [ $\arcsine[(\text{mlgs})^{0.5}]$  versus  $\log(\text{dilution})$ ] to mean largest grain size to limit inherent curvature of dilution plots caused by the "leveling off" of mean largest grain size values for both very dilute and very concentrated thermal hysteresis protein samples.

Claim 20 (currently amended): The recrystallization inhibition assay method as defined in claim 1, wherein linear regression analyses provides the basis for development of a numerical factor representative of said relative recrystallization inhibition ~~Relative Inhibition~~ factor describing the activity of the test solution with respect to recrystallization inhibition capability.

Claim 21 (currently amended): The recrystallization inhibition assay method as defined in claim 20, wherein the relative recrystallization inhibition RI factor is equal to the absolute value of the logarithm of the minimum test solution dilution required to eliminate recrystallization inhibition activity.

Claim 22 (currently amended): The recrystallization inhibition assay method as defined in claim 21, wherein the relative recrystallization inhibition RI factor is a measure of test solution recrystallization inhibition strength, according to the assessed exponential factor required for sufficient dilution of test solution to lose recrystallization inhibition activity, and providing a relative assessment of functional thermal hysteresis concentration within said test solution.

Claim 23 (currently amended): The recrystallization inhibition assay method as defined in claim 21, wherein the relative recrystallization inhibition RI factor provides a relative assessment of functional thermal hysteresis protein concentration, and comparisons of various test solutions concentrations given translational shifts along the X axis showing log(dilution).

Claim 24 (previously amended): The recrystallization inhibition assay method as defined in claim 19, wherein the regression line slope and Y-intercept reflect the recrystallization inhibition potency of a given test solution, thermal hysteresis protein species, recombinant thermal hysteresis protein product, synthetic thermal hysteresis analogue, or the like.

Claim 25 (previously amended): The recrystallization inhibition assay method as defined in claim 19, wherein slope comparisons and shifts along Y-intercept provide relative potency comparisons between test solutions, thermal hysteresis species and the like.

Claim 26 (currently amended): The recrystallization inhibition assay method as defined in claim 20, wherein recrystallization inhibition RI activity of a test solution is expressed as equivalent to the recrystallization inhibition RI activity of a predetermined concentration of Tm 12.86 producing an equivalent recrystallization inhibition RI profile.

Claim 27 (previously amended): The recrystallization inhibition assay method as defined in claim 22, wherein activity and potency of said test solution may include a combination of: more than one type of thermal hysteresis protein, thermal hysteresis protein plus activator solutions such as in test solution of hemolymph, or artificial solutions

containing predetermined amounts of purified thermal hysteresis protein with an activator supplement.

Claim 28 (previously amended): The recrystallization inhibition assay method as defined in claim 1, further comprising mathematical modeling of the recrystallization inhibition process with prediction of effects on slope and Y-intercept and log/log transformations for test solution mean largest grain size data and analysis.

Claim 29 (currently amended): The recrystallization inhibition assay method as defined in claim 1, wherein the relationship between relative recrystallization inhibition (RI) RI factors and thermal hysteresis levels for functionally active test solutions are described by the equation: RI factor =  $1.428 \text{ LOG}(\text{TH}) + 3.703$ .

Claim 30 (previously amended): The recrystallization assay method as defined in claim 1, wherein a random sampling method is used for data collection generating mean largest grain size which significantly eliminates the effect of intrasample ice crystal grain heterogeneity at an annealing temperature in a range of approximately 2 - 4°C below a melting point of said test sample, and with at least one of the following: saline and Phosphate Buffered Saline solvents.

Claim 31 (previously amended): The recrystallization inhibition assay method as defined in claim 1, further used for concurrent multiple sample testing of solutions.

Claim 32 (previously amended): The recrystallization inhibition assay method as defined in claim 31, wherein said multiple testing of solutions includes the "sandwich" method; and application via a 96 well plate device.

Claim 33 (withdrawn): A method for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process as defined in claim 1.

Claim 34 (withdrawn): A method for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process as defined in claim 1.